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1. *What is the purpose of the study?*  
 2. *What are the research objectives?*  
 3. *What is the research methodology?*  
 4. *What are the results of the study?*  
 5. *What are the conclusions of the study?*  
 6. *What are the limitations of the study?*  
 7. *What are the implications of the study?*  
 8. *What are the future research directions?*  
 9. *What are the contributions of the study?*  
 10. *What are the key findings of the study?*  
 11. *What are the main results of the study?*  
 12. *What are the primary outcomes of the study?*  
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124. ANSWER 1 OF 12 HEADLINE COPYRIGHT 1997 ACS  
ADVERTISING NUMBER: 210:2414 HEADLINE  
DOCUMENT NUMBER: 135:207491  
TITLE: Monomeric and dimeric EsRed  
fluorescent protein variants and  
cDNAs and methods for preparing and using the proteins  
INVENTOR(S): Tsien, Roger Y.; Campbell, Robert E.  
PATENT ASSIGNEE(S): USA  
SOURCE: U.S. Pat. Appl. Publ., 67 pp., Cont.-in-part of U.S.  
Pat. Appl. 2003 32,488.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 3  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003/096335	A1	20030327	US 2002/121858	20020419
US 2003/020681	A1	20030215	US 2001/066868	20010311
PRIORITY APLIN. INFO.:			US 2001/084808	20010227
			US 2001/066858	20010524

AB The present invention relates to monomeric and dimeric forms of Anthracene fluorescent proteins, esp. *Vibrio* DsRed. The invention also relates to methods of making such fluorescent protein monomers and dimers using, for example, error-prone PCR. The improved fluorescent proteins may be used for assaying transcription as well as for anal. of in vivo localization or trafficking of proteins. Thus, DsRed variants which are longer tetramers but instead form dimers, or exist as monomers were produced. The monomeric variant mRFP1 matures rapidly and has minimal emission when excited at wavelengths optimal for GFP. Application of antibodies paired with the DsRed system to mutagenesis of GFP results in GFP variants which are larger dimerized.

L2L ANSWER - OF 10 HCAPLUS COPYRIGHT 2003 ACS  
 ACCESSION NUMBER: 2003:114888 HCAPLUS  
 DOCUMENT NUMBER: 137:114888  
 TITLE: **Dimeric fusion proteins as fluorescent proteins showing low levels of oligomerization**  
 INVENTOR S: Tsien, Roger Y.; Campbell, Robert E.  
 PATENT APPLICANT S: USA  
 ADDRESS: U.S. Pat. Appl. 11/111, 48 11/1, 11/11-11/11: 11/11  
 Ser. No. 11/111, 48 11/1  
 C I E N: XXXXX  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY APP. NO. S: 3  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003:114888	A1	2003-11-11	US 2001-866538	2001-11-11
US 2003:114888	A2	2003-11-11	US 2001-866538	2001-11-11
US 2003:114888	A3	2003-11-11		

X: AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC, CD, CE, CF, CG, CH, CI, CJ, CK, CL, CM, CN, CO, CP, CQ, CR, CS, CT, CU, CV, CW, CX, CY, CZ, DA, DB, DC, DD, DE, DF, DG, DH, DI, DJ, DK, DL, DM, DN, DO, DP, DQ, DR, DS, DT, DU, DV, DW, DX, DY, DZ, EA, EB, EC, ED, EE, EF, EG, EH, EI, EJ, EK, EL, EM, EN, EO, EP, EQ, ER, ES, ET, EU, EV, EW, EX, EY, EZ, FA, FB, FC, FD, FE, FF, FG, FH, FI, FJ, FK, FL, FM, FN, FO, FP, FQ, FR, FS, FT, FU, FV, FW, FX, FY, FZ, GA, GB, GC, GD, GE, GF, GG, GH, GI, GJ, GK, GL, GM, GN, GO, GP, GQ, GR, GS, GT, GU, GV, GW, GX, GY, GZ, HA, HB, HC, HD, HE, HF, HG, HH, HI, HJ, HK, HL, HM, HN, HO, HP, HQ, HR, HS, HT, HU, HV, HW, HX, HY, HZ, IA, IB, IC, ID, IE, IF, IG, IH, II, IJ, IK, IL, IM, IN, IO, IP, IQ, IR, IS, IT, IU, IV, IW, IX, IY, IZ, JA, JB, JC, JD, JE, JF, JG, JH, JI, JJ, JK, JL, JM, JN, JO, JP, JQ, JR, JS, JT, JU, JV, JW, JX, JY, JZ, KA, KB, KC, KD, KE, KF, KG, KH, KI, KJ, KK, KL, KM, KN, KO, KP, KQ, KR, KS, KT, KU, KV, KW, KY, KZ, LA, LB, LC, LD, LE, LF, LG, LH, LI, LJ, LK, LL, LM, LN, LO, LP, LQ, LR, LS, LT, LU, LV, LW, LX, LY, LZ, MA, MB, MC, MD, ME, MF, MG, MH, MI, MJ, MK, ML, MN, MO, MP, MQ, MR, MS, MT, MU, MV, MW, MX, MY, MZ, NA, NB, NC, ND, NE, NF, NG, NH, NI, NJ, NK, NL, NM, NO, NP, NQ, NR, NS, NT, NU, NV, NW, NX, NY, NZ, OA, OB, OC, OD, OE, OF, OG, OH, OI, OJ, OK, OL, OM, ON, OO, OP, OQ, OR, OS, OT, OU, OV, OW, OX, OY, OZ, PA, PB, PC, PD, PE, PF, PG, PH, PI, PJ, PK, PL, PM, PN, PO, PP, PQ, PR, PS, PT, PU, PV, PW, PX, PY, PZ, QA, QB, QC, QD, QE, QF, QG, QH, QI, QJ, QK, QL, QM, QN, QO, QP, QQ, QR, QS, QT, QU, QV, QW, QX, QY, QZ, RA, RB, RC, RD, RE, RF, RG, RH, RI, RJ, RK, RL, RM, RN, RO, RP, RQ, RR, RS, RT, RU, RV, RW, RX, RY, RZ, SA, SB, SC, SD, SE, SF, SG, SH, SI, SJ, SK, SL, SM, SN, SO, SP, SQ, SR, SS, ST, SU, SV, SW, SX, SY, SZ, TA, TB, TC, TD, TE, TF, TG, TH, TI, TJ, TK, TL, TM, TN, TO, TP, TQ, TR, TS, TT, TU, TV, TW, TX, TY, TZ, UA, UB, UC, UD, UE, UF, UG, UH, UI, UJ, UK, UL, UM, UN, UO, UP, UQ, UR, US, UT, UV, UW, UX, UY, UZ, VA, VB, VC, VD, VE, VF, VG, VH, VI, VJ, VK, VL, VM, VN, VO, VP, VQ, VR, VS, VT, VU, VV, VW, VX, VY, VZ, WA, WB, WC, WD, WE, WF, WG, WH, WI, WJ, WK, WL, WM, WN, WO, WP, WQ, WR, WS, WT, WU, WV, WW, WX, WY, WZ, XA, XB, XC, XD, XE, XF, XG, XH, XI, XJ, XK, XL, XM, XN, XO, XP, XQ, XR, XS, XT, XU, XV, XW, XX, XY, XZ, YA, YB, YC, YD, YE, YF, YG, YH, YI, YJ, YK, YL, YM, YN, YO, YP, YQ, YR, YS, YT, YU, YV, YW, YX, YY, YZ, ZA, ZB, ZC, ZD, ZE, ZF, ZG, ZH, ZI, ZJ, ZK, ZL, ZM, ZN, ZO, ZP, ZQ, ZR, ZS, ZT, ZU, ZV, ZW, ZX, ZY, ZZ.

US 2003:114888 A1 20030327 US 2002-121258 20020410  
 PRIORITY APPLN. INFO.: US 2001-794308 A2 20010226  
 US 2001-866538 A 20010524

AB Fluorescent proteins, such as **green fluorescent protein**, that show lower degrees of oligomerization are described for anal. use. The non-oligomerizing derivs. are **fusion proteins** of **fluorescent proteins** including a naturally occurring **green fluorescent protein**, a **red fluorescent protein** or derivs. that are **dimers** of the **fluorescent protein** connected by a linker peptide. The protein may also carry other modifications, such as in surface residues, that prevent oligomerization without a significant impact on fluorescence properties. Also provided is a fusion protein, which includes a non-oligomerizing fluorescent protein linked to at least one polypeptide of interest. Chimeric genes encoding these fusion proteins and expression constructs and hosts for the genes are also described.

L2L ANSWER - OF 10 HCAPLUS COPYRIGHT 2003 ACS  
 ACCESSION NUMBER: 2003:114888 HCAPLUS  
 DOCUMENT NUMBER: 137:114888  
 TITLE: **Non-oligomerizing fluorescent proteins and their uses**  
 INVENTOR S: Tsien, Roger Y.; Baird, Geoffrey J.;  
 Campbell, Robert E.; Zacharias, David A.  
 PATENT APPLICANT S: The Regents of the University of California, USA  
 ADDRESS: U.S. Pat. Appl. 11/111, 48 11/1  
 C I E N: XXXXX  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English



Run: 10/10/10

Journal code: 0360-5310. ISSN: 0360-5310.  
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ORIG. SOURCE: GENBANK-APF 0.01; GENBANK-APF 0.01; GENBANK-APF 0.01  
ENTRY NUMBER: 100000  
ENTRY DATE: Entered STN: 10/10/10  
Last Updated on STN: 10/10/10  
Entered Medline: 10/10/10

AB All monomeric fluorescent proteins almost always display some form of quaternary structure, including the weak tendency to aggregate. **green fluorescent protein (GFP)** dimerize, the relative dimerization of **benilla GFP**, and the relative tetramerization of the red fluorescent protein **triple red fluorescent protein**. Although the weak dimerization of **benilla GFP** has not impaired its acceptance as an indispensable tool in cell biology, the relative tetramerization of **benilla** has greatly hindered its use as a genetically encoded fusion tag. We present here the stepwise evolution of **benilla** to a dimer and then either to a genetic fusion of two copies of the protein, i.e., a tandem dimer, or to a true monomer designated **mRFP1** (monomeric red fluorescent protein). Each subunit interface was disrupted by insertion of amines, which initially crippled the resulting protein, but the resulting **mRFP1** could be rescued by random and directed mutagenesis resulting in **mRFP1** variants in the dimer and in **mRFP1**. Fusion of the **mRFP1** function protein **connexin** to **mRFP1** rendered fully functional. **mRFP1**, which is analogous to the tetramer and dimer **benilla**. Although **mRFP1** has somewhat lower extinction coefficient, quantum yield, and photostability than **benilla**, **mRFP1** matures 10 times faster, so that it shows similar brightness in living cells. In addition, the excitation and emission peaks of **mRFP1**, 584 and 607 nm, are approximately 25 nm red-shifted from **benilla**, which should confer greater tissue penetration and spectral separation from autofluorescence and other fluorescent proteins.

L2L ANSWER 8 OF 10 MEDLINE DUPLICATE 2  
ACCESSION NUMBER: 12349561 MEDLINE  
COMMENT NUMBER: 1044700 PubMed ID: 11288376  
TITLE: Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells.  
COMMENT: Comment in: Science. 2002 May 3;296(5569):913-6.  
AUTHOR: Zacharias David A; Violin Jonathan B; Newton Alexandra C; Tsien Roger Y  
CORPORATE SOURCE: Department of Pharmacology, Biomedical Sciences Graduate Program, and, Howard Hughes Medical Institute, University of California, San Diego, La Jolla, CA 92093-0647, USA.  
CONTRACT NUMBER: 1234567890 NIGMS  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE  
LANGUAGE: English  
FILE COMMENT: Priority Journals  
ENTRY NUMBER: 100000  
ENTRY DATE: Entered STN: 10/10/10  
Last Updated on STN: 10/10/10  
Entered Medline: 10/10/10

AB Many proteins associated with the plasma membrane are known to partition

Figure 1. The effect of the concentration of the *Agaricus bisporus* spores on the growth of *Agaricus bisporus* and *Agaricus bisporus* spores. The concentration of the spores was 10<sup>6</sup> spores/ml (A), 10<sup>7</sup> spores/ml (B), 10<sup>8</sup> spores/ml (C), and 10<sup>9</sup> spores/ml (D). The concentration of the spores was 10<sup>6</sup> spores/ml (A), 10<sup>7</sup> spores/ml (B), 10<sup>8</sup> spores/ml (C), and 10<sup>9</sup> spores/ml (D).

[illegible]

**PROOF:**      Let  $a = \frac{m}{n}$ ,  $b = \frac{r}{s}$ . Then  $\frac{a+b}{2} = \frac{\frac{m}{n} + \frac{r}{s}}{2} = \frac{\frac{ms+rn}{ns}}{2} = \frac{ms+rn}{2ns}$ .

Let  $c = \frac{ms+rn}{2ns}$ . Then  $c$  is between  $a$  and  $b$ .  
We have  $\frac{a+c}{2} = \frac{\frac{m}{n} + \frac{ms+rn}{2ns}}{2} = \frac{\frac{2ms+rn}{2ns}}{2} = \frac{2ms+rn}{4ns}$ .

Let  $d = \frac{2ms+rn}{4ns}$ . Then  $d$  is between  $a$  and  $c$ .  
We have  $\frac{a+d}{2} = \frac{\frac{m}{n} + \frac{2ms+rn}{4ns}}{2} = \frac{\frac{4ms+2rn}{4ns}}{2} = \frac{4ms+2rn}{8ns}$ .

Let  $e = \frac{4ms+2rn}{8ns}$ . Then  $e$  is between  $a$  and  $d$ .  
We have  $\frac{a+e}{2} = \frac{\frac{m}{n} + \frac{4ms+2rn}{8ns}}{2} = \frac{\frac{8ms+4rn}{8ns}}{2} = \frac{8ms+4rn}{16ns}$ .

Let  $f = \frac{8ms+4rn}{16ns}$ . Then  $f$  is between  $a$  and  $e$ .  
We have  $\frac{a+f}{2} = \frac{\frac{m}{n} + \frac{8ms+4rn}{16ns}}{2} = \frac{\frac{16ms+8rn}{16ns}}{2} = \frac{16ms+8rn}{32ns}$ .

Let  $g = \frac{16ms+8rn}{32ns}$ . Then  $g$  is between  $a$  and  $f$ .  
We have  $\frac{a+g}{2} = \frac{\frac{m}{n} + \frac{16ms+8rn}{32ns}}{2} = \frac{\frac{32ms+16rn}{32ns}}{2} = \frac{32ms+16rn}{64ns}$ .

Let  $h = \frac{32ms+16rn}{64ns}$ . Then  $h$  is between  $a$  and  $g$ .  
We have  $\frac{a+h}{2} = \frac{\frac{m}{n} + \frac{32ms+16rn}{64ns}}{2} = \frac{\frac{64ms+32rn}{64ns}}{2} = \frac{64ms+32rn}{128ns}$ .

Let  $i = \frac{64ms+32rn}{128ns}$ . Then  $i$  is between  $a$  and  $h$ .  
We have  $\frac{a+i}{2} = \frac{\frac{m}{n} + \frac{64ms+32rn}{128ns}}{2} = \frac{\frac{128ms+64rn}{128ns}}{2} = \frac{128ms+64rn}{256ns}$ .

Let  $j = \frac{128ms+64rn}{256ns}$ . Then  $j$  is between  $a$  and  $i$ .  
We have  $\frac{a+j}{2} = \frac{\frac{m}{n} + \frac{128ms+64rn}{256ns}}{2} = \frac{\frac{256ms+128rn}{256ns}}{2} = \frac{256ms+128rn}{512ns}$ .

Let  $k = \frac{256ms+128rn}{512ns}$ . Then  $k$  is between  $a$  and  $j$ .  
We have  $\frac{a+k}{2} = \frac{\frac{m}{n} + \frac{256ms+128rn}{512ns}}{2} = \frac{\frac{512ms+256rn}{512ns}}{2} = \frac{512ms+256rn}{1024ns}$ .

Let  $l = \frac{512ms+256rn}{1024ns}$ . Then  $l$  is between  $a$  and  $k$ .  
We have  $\frac{a+l}{2} = \frac{\frac{m}{n} + \frac{512ms+256rn}{1024ns}}{2} = \frac{\frac{1024ms+512rn}{1024ns}}{2} = \frac{1024ms+512rn}{2048ns}$ .

Let  $m = \frac{1024ms+512rn}{2048ns}$ . Then  $m$  is between  $a$  and  $l$ .  
We have  $\frac{a+m}{2} = \frac{\frac{m}{n} + \frac{1024ms+512rn}{2048ns}}{2} = \frac{\frac{2048ms+1024rn}{2048ns}}{2} = \frac{2048ms+1024rn}{4096ns}$ .

Let  $n = \frac{2048ms+1024rn}{4096ns}$ . Then  $n$  is between  $a$  and  $m$ .  
We have  $\frac{a+n}{2} = \frac{\frac{m}{n} + \frac{2048ms+1024rn}{4096ns}}{2} = \frac{\frac{4096ms+2048rn}{4096ns}}{2} = \frac{4096ms+2048rn}{8192ns}$ .

Let  $p = \frac{4096ms+2048rn}{8192ns}$ . Then  $p$  is between  $a$  and  $n$ .  
We have  $\frac{a+p}{2} = \frac{\frac{m}{n} + \frac{4096ms+2048rn}{8192ns}}{2} = \frac{\frac{8192ms+4096rn}{8192ns}}{2} = \frac{8192ms+4096rn}{16384ns}$ .

Let  $q = \frac{8192ms+4096rn}{16384ns}$ . Then  $q$  is between  $a$  and  $p$ .  
We have  $\frac{a+q}{2} = \frac{\frac{m}{n} + \frac{8192ms+4096rn}{16384ns}}{2} = \frac{\frac{16384ms+8192rn}{16384ns}}{2} = \frac{16384ms+8192rn}{32768ns}$ .

Let  $r = \frac{16384ms+8192rn}{32768ns}$ . Then  $r$  is between  $a$  and  $q$ .  
We have  $\frac{a+r}{2} = \frac{\frac{m}{n} + \frac{16384ms+8192rn}{32768ns}}{2} = \frac{\frac{32768ms+16384rn}{32768ns}}{2} = \frac{32768ms+16384rn}{65536ns}$ .

Let  $s = \frac{32768ms+16384rn}{65536ns}$ . Then  $s$  is between  $a$  and  $r$ .  
We have  $\frac{a+s}{2} = \frac{\frac{m}{n} + \frac{32768ms+16384rn}{65536ns}}{2} = \frac{\frac{65536ms+32768rn}{65536ns}}{2} = \frac{65536ms+32768rn}{131072ns}$ .

Let  $t = \frac{65536ms+32768rn}{131072ns}$ . Then  $t$  is between  $a$  and  $s$ .  
We have  $\frac{a+t}{2} = \frac{\frac{m}{n} + \frac{65536ms+32768rn}{131072ns}}{2} = \frac{\frac{131072ms+65536rn}{131072ns}}{2} = \frac{131072ms+65536rn}{262144ns}$ .

Let  $u = \frac{131072ms+65536rn}{262144ns}$ . Then  $u$  is between  $a$  and  $t$ .  
We have  $\frac{a+u}{2} = \frac{\frac{m}{n} + \frac{131072ms+65536rn}{262144ns}}{2} = \frac{\frac{262144ms+131072rn}{262144ns}}{2} = \frac{262144ms+131072rn}{524288ns}$ .

Let  $v = \frac{262144ms+131072rn}{524288ns}$ . Then  $v$  is between  $a$  and  $u$ .  
We have  $\frac{a+v}{2} = \frac{\frac{m}{n} + \frac{262144ms+131072rn}{524288ns}}{2} = \frac{\frac{524288ms+262144rn}{524288ns}}{2} = \frac{524288ms+262144rn}{1048576ns}$ .

Let  $w = \frac{524288ms+262144rn}{1048576ns}$ . Then  $w$  is between  $a$  and  $v$ .  
We have  $\frac{a+w}{2} = \frac{\frac{m}{n} + \frac{524288ms+262144rn}{1048576ns}}{2} = \frac{\frac{1048576ms+524288rn}{1048576ns}}{2} = \frac{1048576ms+524288rn}{2097152ns}$ .

Let  $x = \frac{1048576ms+524288rn}{2097152ns}$ . Then  $x$  is between  $a$  and  $w$ .  
We have  $\frac{a+x}{2} = \frac{\frac{m}{n} + \frac{1048576ms+524288rn}{2097152ns}}{2} = \frac{\frac{2097152ms+1048576rn}{2097152ns}}{2} = \frac{2097152ms+1048576rn}{4194304ns}$ .

Let  $y = \frac{2097152ms+1048576rn}{4194304ns}$ . Then  $y$  is between  $a$  and  $x$ .  
We have  $\frac{a+y}{2} = \frac{\frac{m}{n} + \frac{2097152ms+1048576rn}{4194304ns}}{2} = \frac{\frac{4194304ms+2097152rn}{4194304ns}}{2} = \frac{4194304ms+2097152rn}{8388608ns}$ .

Let  $z = \frac{4194304ms+2097152rn}{8388608ns}$ . Then  $z$  is between  $a$  and  $y$ .  
We have  $\frac{a+z}{2} = \frac{\frac{m}{n} + \frac{4194304ms+2097152rn}{8388608ns}}{2} = \frac{\frac{8388608ms+4194304rn}{8388608ns}}{2} = \frac{8388608ms+4194304rn}{16777216ns}$ .

Let  $A = \frac{8388608ms+4194304rn}{16777216ns}$ . Then  $A$  is between  $a$  and  $z$ .  
We have  $\frac{a+A}{2} = \frac{\frac{m}{n} + \frac{8388608ms+4194304rn}{16777216ns}}{2} = \frac{\frac{16777216ms+8388608rn}{16777216ns}}{2} = \frac{16777216ms+8388608rn}{33554432ns}$ .

Let  $B = \frac{16777216ms+8388608rn}{33554432ns}$ . Then  $B$  is between  $a$  and  $A$ .  
We have  $\frac{a+B}{2} = \frac{\frac{m}{n} + \frac{16777216ms+8388608rn}{33554432ns}}{2} = \frac{\frac{33554432ms+16777216rn}{33554432ns}}{2} = \frac{33554432ms+16777216rn}{67108864ns}$ .

Let  $C = \frac{33554432ms+1677$

ATTACHED: Copy 1, Exhibit 1; Brown, Stephen G.; Davidson, Christopher M.; Campbell, R. Duncan (1)

CORPORATE ADDRESS: 1 Functional Genomics Group, MRC UK HRI Wellcome Centre,  
Bluntell, Cambridge, CB1 6BX; e-mail: hannah.barnes@hrc.ac.uk

NOTE: For chemical names, 1 February, 1991, Vol. 1, No. 1, pp. 44-46, <http://www.fic.nyu.edu/print>.  
DOI: 10.1002/1522

1. *Chlorophyll a* and *Chlorophyll b* were determined by the method of Arar and Collins (1971) using a Shimadzu 1010 spectrophotometer.

1. *Journal of the American Medical Association*, 1997; 277: 1001-1005.

AB The inhibitory kappaB (IkappaB)-like (IkappaBL) gene is located within the class III region of the MHC on human chromosome 6. Previous analysis of the predicted amino acid sequence of the human IkappaBL protein revealed three putative functional domains; 1-3 ankyrin repeat sequences, which are similar to the second and third ankyrin repeats of the nuclear factor kappaB (NF-kappaB) protein; three FESF sequence motifs (a sequence that is rich in proline, serine, aspartic acid and threonine residues), which are also found in other IkappaB family members; and a C-terminal leucine zipper-like motif. In the present study we have identified a novel bipartite motif, which is required for nuclear localization of the IkappaBL protein. Analyses of IkappaBL-specific transcripts revealed the existence of a widely expressed spliced variant form: IkappaBL1 (IkappaBL $\Delta$ 1), which lacks the amino acid sequence **SELESTWVHWKRTF** where single-letter amino-acid notation has been used. Interestingly, transfection of IkappaBL mRNA in vivo was found to initiate predominantly from the second available methionine, thereby resulting in the disruption of the predicted N-terminal FESF sequence. Also, transient expression of T3 epitope-tagged IkappaBL and IkappaBL $\Delta$ 1 proteins in mammalian cells showed that both proteins were targeted to the nucleus, where they accumulate in nuclear speckles. To define the protein domains required for nuclear import and subnuclear localization, a complementary set of deletion mutants and enhanced green fluorescent protein-IkappaBL domain fusions were expressed in mammalian cells. Data from these experiments show that a combination of the ankyrin-repeat region and an adjacent arginine-rich sequence are necessary and sufficient for both nuclear import and speckle localization.

1. The first step is to identify the problem. This involves understanding the current situation and what needs to be improved.

1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463, 2464, 2465, 2466, 2467, 2468, 2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481, 2482, 2483, 2484, 2485, 2486, 2487, 2488, 2489, 2490, 2491, 2492, 2493, 2494, 2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2511, 2512, 2513, 2514, 2515, 2516, 2517, 2518, 2519, 2520, 2521, 2522, 2523, 2524, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2533, 2534, 2535, 2536, 2537, 2538, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2548, 2549, 2550, 2551, 2552, 2553, 2554, 2555, 2556, 2557, 2558, 2559, 2560, 2561, 2562, 2563, 2564, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2572, 2573, 2574, 2575, 2576, 2577, 2578, 2579, 2580, 2581, 2582, 2583, 2584, 2585, 2586, 2587, 2588, 2589, 2590, 2591, 2592, 2593, 2594, 2595, 2596, 2597, 2598, 2599, 2600, 2601, 2602, 2603, 2604, 2605, 2606, 2607, 2608, 2609, 2610, 2611, 2612, 2613, 2614, 2615, 2616, 2617, 2618, 2619, 2620, 2621, 2622, 2623, 2624, 2625, 2626, 2627, 2628, 2629, 2630, 2631, 2632, 2633, 2634, 2635, 2636, 2637, 2638, 2639, 2640, 2641, 2642, 2643, 2644, 2645, 2646, 2647, 2648, 2649, 2650, 2651, 2652, 2653, 2654, 2655, 2656, 2657, 2658, 2659, 2660, 2661, 2662, 2663, 2664, 2665, 2666, 2667, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 2676, 2677, 2678, 2679, 26

```

# Create a new plot
plot(
  # The data
  data,
  # The variable to plot
  green ~ blue,
  # The variable to facet by
  facet ~ red
)

```

Tsien, Roger Y.; Hsiao, Hsueh-

PATENT ASSIGNEE: Regents of the University of California, USA  
 SOURCE: U.S., 20 Apr., Cont.-in-part of U.S. 5,411,441.  
 INVENTOR: TSUEN  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY APP. NO.: 3  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5,411,441	B1	21 11 12	US 1997-074781	1997 11 12
US 5,411,441	A	1997 04 29	US 1994-337915	1994 11 10
CA 2343816	AA	1998 08 08	CA 1995-2343816	1995 11 13
WO 9623816	A1	1996 08 08	WO 1995-US14692	1995 11 13

K: AL, AM, AN, AO, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GR, HU, IS, JP, KE, KG, KP, KR, KZ, LA, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SE, SG, SI, SK

EW: KE, LS, MK, SD, SZ, TG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, NL, NL, PT, SE, BF, EC, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

EP 114709	A2	2001 06 06	EP 2001-105011	1995 11 13
EP 114709	A3	2002 09 18		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE, MC, PT, IE

US 5,411,441	A	1998 07 07	US 1996-753143	1996 11 13
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PRIORITY APPL. INFO.:

US 1994-337915	AL	1994 11 10
WO 1995-US14692	W	1995 11 13
CA 1995-2343816	A3	1995 11 13
EP 1995-939898	A3	1995 11 13
US 1996-727452	A3	1996 10 16

AB Modifications in the sequence of Aequorea wild-type GFP provide products having markedly different excitation and emission spectra from corresponding products from wild-type GFP. In one class of modifications, the product derived from the modified GFP exhibits an alteration in the ratio of two main excitation peaks obsd. with the product derived from wild-type GFP. In another class, the product derived from the modified GFP fluoresces at a shorter wavelength than the corresponding product from wild-type GFP. In yet another class of modifications, the product derived from the modified GFP exhibits only a single excitation peak and enhanced emission relative to the product derived from wild-type GFP.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER # OF 13 MEDLINE DUPLICATE 3  
 ACCESSION NUMBER: 2001429679 MEDLINE  
 DOCUMENT NUMBER: 21369963 PubMed ID: 11387331  
 TITLE: Reducing the environmental sensitivity of yellow fluorescent protein. Mechanism and applications.  
 AUTHOR: Griesbeck O; Baird S S; Campbell R E; Zacharias D A; Tsien R Y  
 CORPORATE SOURCE: Howard Hughes Medical Institute, University of California, San Diego, La Jolla, California 92093-0647, USA.  
 CONTRACT NUMBER: EP/CA23100-16 NCII  
 NO-17177 NINDS  
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, 2001 Apr; 276 (31): 27194-27194.  
 Journal Code: 2001111R. ISSN: 0721-9241.

Run: 1/1/97, 1/1/97

ENTRY NUMBER: United States  
DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE  
LANGUAGE: English  
FILE SEGMENT: Library Journals  
JOURN REF NO: 110-1100  
ENTRY MONTH: 1/1/97  
ENTRY DATE: Entered: JTN: 1/1/97  
Last Updated in JTN: 1/1/97  
Entered: Modified: 1/1/97

AB The development of the **green** fluorescent protein (YFP) as a genetic marker has led to a variety of applications in signal transduction and biology to monitor protein-protein interactions. However, previous YFPs show excessive pH sensitivity, chloride interference, poor photostability, and poor expression at 37 degrees C. protein evolution in *Escherichia coli* has produced a new one named **Urine**, in which the mutation GFPN confers a much lower pKa (6.7) than for previous YFPs, indifference to chloride, twice the photostability of previous YFPs, and much better expression at 37 degrees C and in *transfected*. The chloride resistance is explained by a 3.0-A x-ray crystal structure of **Urine**, showing that the methionine side chain fills what was once a large chloride-binding cavity adjacent to the chromophore. Insertion of calmodulin within **Urine** as a **fusion of YFP fluorescent protein, calmodulin, a calmodulin-binding** peptide, and **Urine** has generated improved calcium indicators. These indicators can be targeted to multiple cellular locations and have permitted the first single-cell imaging of free  $Ca^{2+}$  in the *algae*. **Urine** is superior to all previous YFPs except when pH or chloride sensitivity is desired and is particularly advantageous within genetically encoded fluorescent indicators of physiological signals.

LEA ANSWER: OF 12 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 2001:657326 HCAPLUS  
DOCUMENT NUMBER: 136:321466  
TITLE: Fluorescence resonance energy transfer analysis of cell surface receptor interactions and signaling using spectral variants of the **green** fluorescent protein  
AUTHOR(S): Chan, Francis Ka-Ming; Siegel, Richard M.; Zacharias, David; Swatford, Ruth; Holmes, Kevin L.; Tsien, Roger Y.; Lenardo, Michael J.  
CORRELATE SOURCE: Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892-1492, USA  
SOURCE: Cytometry (2001), 44(4), 361-368  
CODEN: CYTODQ; ISSN: 0196-4763  
PUBLISHER: Wiley-Liss, Inc.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Fluorescence resonance energy transfer (FRET) is a powerful technique for measuring mol. interactions at Angstrom distances. We present a new method for FRET that utilizes the unique spectral properties of variants of the **green** fluorescent protein (GFP) for large-scale analysis of cell cytometry. The proteins of interest are fused in frame, sep., to the cyan fluorescent protein (CFP) or the yellow fluorescent protein (YFP). FRET between these differentially tagged fusion proteins is analyzed using a dual-laser FACS Advantage cytometer. We show that homotypic interactions between individual receptor chains of tumor necrosis factor receptor (TNFR) family members can be detected as FRET from CFP-tagged receptor chains to YFP-tagged receptor chains. Noncovalent mol.

intracellular and extracellular regions of the receptor chains. The specificity of the assay is demonstrated by the absence of FRET between hetero-oligomer pairs that do not interact with each other. Interaction between a TNFR-like receptor (p55) and a membrane-associated protein (CD40) is demonstrated by FRET. The ability of several variants of GFP to allow cytofluorescence FRET analysis of membrane receptors is demonstrated. This method of analyzing FRET allows probing of intracellular interactions that involve both the intracellular and extracellular regions of membrane proteins as well as proteins within the cells. Unlike other methods, FRET allows the quant. determination of the level of interaction in living cells. Moreover, flow cytometry allows quant. analyses to be carried out on a cell-by-cell basis in large cell populations.

REFERENCE COUNT: 13 THERE ARE 25 OTHER REFERENCES AVAILABLE FOR THIS REPORT. ALL CITATIONS AVAILABLE IN THE REF. COUNT

ALL ANSWERS TO E-MAIL: E-MAIL: COPYRIGHT 1998 AND

APPROPRIATE NUMBER: 1998-42116 E-MAIL:

1998-42116 E-MAIL:

TITLE: Green fluorescent protein analysis containing ligand-binding sites and peptides for use as reporter molecules

INVENTOR(S): Tsien, Roger Y.; Baird, Geoffrey A.

PATENT APPLICATION NO.: 1998-42116 E-MAIL:

CLASS: 1998-42116 E-MAIL:

CODEN: FIMX02

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY AND NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 1998-01116	A1	20001116	WO 1998-01116	20000617
WO 1998-01116	A2	20002074		
WO 1998-01116	A3	20002074		
WO 1998-01116	A4	20002074		
WO 1998-01116	A5	20002074		
WO 1998-01116	A6	20002074		
WO 1998-01116	A7	20002074		
WO 1998-01116	A8	20002074		
WO 1998-01116	A9	20002074		
WO 1998-01116	A10	20002074		
WO 1998-01116	A11	20002074		
WO 1998-01116	A12	20002074		
WO 1998-01116	A13	20002074		
WO 1998-01116	A14	20002074		
WO 1998-01116	A15	20002074		
WO 1998-01116	A16	20002074		
WO 1998-01116	A17	20002074		
WO 1998-01116	A18	20002074		
WO 1998-01116	A19	20002074		
WO 1998-01116	A20	20002074		
WO 1998-01116	A21	20002074		
WO 1998-01116	A22	20002074		
WO 1998-01116	A23	20002074		
WO 1998-01116	A24	20002074		
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WO 1998-01116	A26	20002074		
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WO 1998-01116	A30	20002074		
WO 1998-01116	A31	20002074		
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WO 1998-01116	A34	20002074		
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WO 1998-01116	A36	20002074		
WO 1998-01116	A37	20002074		
WO 1998-01116	A38	20002074		
WO 1998-01116	A39	20002074		
WO 1998-01116	A40	20002074		
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WO 1998-01116	A42	20002074		
WO 1998-01116	A43	20002074		
WO 1998-01116	A44	20002074		
WO 1998-01116	A45	20002074		
WO 1998-01116	A46	20002074		
WO 1998-01116	A47	20002074		
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WO 1998-01116	A51	20002074		
WO 1998-01116	A52	20002074		
WO 1998-01116	A53	20002074		
WO 1998-01116	A54	20002074		
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WO 1998-01116	A57	20002074		
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WO 1998-01116	A59	20002074		
WO 1998-01116	A60	20002074		
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WO 1998-01116	A62	20002074		
WO 1998-01116	A63	20002074		
WO 1998-01116	A64	20002074		
WO 1998-01116	A65	20002074		
WO 1998-01116	A66	20002074		
WO 1998-01116	A67	20002074		
WO 1998-01116	A68	20002074		
WO 1998-01116	A69	20002074		
WO 1998-01116	A70	20002074		
WO 1998-01116	A71	20002074		
WO 1998-01116	A72	20002074		
WO 1998-01116	A73	20002074		
WO 1998-01116	A74	20002074		
WO 1998-01116	A75	20002074		
WO 1998-01116	A76	20002074		
WO 1998-01116	A77	20002074		
WO 1998-01116	A78	20002074		
WO 1998-01116	A79	20002074		
WO 1998-01116	A80	20002074		
WO 1998-01116	A81	20002074		
WO 1998-01116	A82	20002074		
WO 1998-01116	A83	20002074		
WO 1998-01116	A84	20002074		
WO 1998-01116	A85	20002074		
WO 1998-01116	A86	20002074		
WO 1998-01116	A87	20002074		
WO 1998-01116	A88	20002074		
WO 1998-01116	A89	20002074		
WO 1998-01116	A90	20002074		
WO 1998-01116	A91	20002074		
WO 1998-01116	A92	20002074		
WO 1998-01116	A93	20002074		
WO 1998-01116	A94	20002074		
WO 1998-01116	A95	20002074		
WO 1998-01116	A96	20002074		
WO 1998-01116	A97	20002074		
WO 1998-01116	A98	20002074		
WO 1998-01116	A99	20002074		
WO 1998-01116	A100	20002074		

PRIORITY APPLICATION NO.:

US 1998-316919 A 1998-04-01  
US 1998-316920 A 1998-04-01  
WO 1998-01116 W 1998-04-01

AB The present invention provides polypeptide and polynucleotide encoding fluorescent indicators having inserted within a fluorescent moiety a gene for polypeptide. The proteins are derived that are not normally fluorescent as a result of FRET coupling. Binding of a ligand to the sensor results in a conformational change and an increase in fluorescence of the protein. Also provided are methods of using the fluorescent indicators. Directly promoted fluorescent polypeptides and



PATIENT NO.	SEX	AGE	APPLICATION NO.	DATE
100-100001	A	20-29	100-100001-001	1990-01-01
100-100002	A	30-39	100-100002-001	1990-02-01
100-100003	A	40-49	100-100003-001	1990-03-01
100-100004	A	50-59	100-100004-001	1990-04-01

LET ANSWER IN QF 19 HCAPLUS COPYRIGHT 2014 ACS  
ACCESSION NUMBER: 12000140115 HCAPLUS  
DOCUMENT NUMBER: 121101-425  
TITLE: A genetically encoded, fluorescent indicator for  
cytosolic AMP in living cells  
AUTHOR(S): Saccoccia, Mannello; Di Girolamo, Francesca; Cho, Charles  
Y.; Feng, Luxin; Knapp, Tim; Neumlescu, Paul A.;  
Taylor, Susan J.; Tsien, Roger Y.; Pannas,  
Thilo  
CORRESPONDENCE: Department of Experimental Biomedical Sciences,  
University of Padova, Padova, 35131, Italy  
SOURCE: Nature Cell Biology (2006), Vol. 8, 15-24  
URI: NCBI/NL; ISSN: 1474-7601  
PUBLISHER: Humana Press

[illegible]

AB The GMP controls several signalling cascades within cells, and therefore in the area of this sector research have an essential role in many cellular events. Here we describe a new method, for monitoring the fluctuations of GMP in living cells. By fusing the GMP-sensor protein kinase A with the variable **green** fluorescent protein epitope, we have obtained a probe in which the fluorescent resonance energy transfer between the fluorescent epitope is dependent on the levels of GMP. This method will point the way to the elucidation of the role of GMP in life.

RECEIVED 11/10/74. THE INFORMATION REFERRED TO IN THIS REPORT, ALL DETAILS AVAILABLE IN THE REPORT

[illegible]

— *Journal of the American Medical Association*, 1997; 277: 1025-1028

TITLE: HEPATOCYTE ENZYME ACTIVITY BY PRODUCTION OF GREEN  
FLUORESCENT PROTEIN EXPRESSED IN THE LIVER OF SYNGENE  
MICE.

Authors: Hsu, S. C.; Hsu, S. C.; Hsu, S. C.; Tsien, R. W.

2000年12月29日，在“2000年中国最佳新闻”评选中，新华社北京29日电《中国首任驻外大使在任期间去世》一文，荣获“2000年中国最佳新闻”称号。

DATE: 11/15/78 BY: JAMES H. HARRIS, JR. 11/26/78

[illegible]

$\frac{d}{dt} \left( \frac{\partial L}{\partial \dot{x}} \right) = \frac{\partial L}{\partial x}$

[illegible][illegible][illegible][illegible]

SUBJECTS: 100; AGE RANGE: 18-65.

AB FM dyes allow fluorescence imaging of active presynaptic terminals and provide valuable insights into dynamics of vesicular turnover, but are not ideally suited for studying the kinetics of exocytosis. FM1-43, for example, requires approx 5 s to depart from the membrane. To selectively monitor exocytosis independent of endocytosis we have developed a new approach based on the quenching of a fluorophore permanently held within the vesicle lumen by a probe that enters vesicles upon fusion. The fluorophore is green fluorescent protein (GFP) associated with the luminal domain of VAMP (synaptobrevin-2), introduced by transfection, and the quencher is promophenol blue (PPB), a small, hydrophilic molecule. In quenching experiments, boutons were first exposed to PPB in  $\text{Ca}^{2+}$ -free Tyrode to eliminate fluorescence of any VAMP-GFP that had somehow been stranded on the plasma membrane. Subsequent exposure to 2 mM  $\text{K}^{+}$ /2 mM  $\text{Ca}^{2+}$  to induce voltage-gated  $\text{Ca}^{2+}$  entry caused sharp drop in GFP fluorescence ( $n=7$ ), with biphasic kinetics reminiscent of restoration of FM1-43. In contrast, high  $\text{K}^{+}$ /2 mM  $\text{Ca}^{2+}$  stimulation in the absence of PPB failed to decrease the fluorescence of puncta, even though a later repeat of the same challenge in PPB caused clear decreases in fluorescence in the same puncta ( $n=8$ ). Evidently, pH increases during exocytosis had no significant effect on the fluorescence of this GFP construct. When PPB-loaded boutons were subjected to high  $\text{K}^{+}$ /2 mM  $\text{Ca}^{2+}$  stimulation in the absence of PPB, the fluorescence of puncta suddenly increased next, as expected if PPB had escaped from vesicles undergoing fusion. One advantage of this approach is that VAMP-GFP can mark boutons even if they are functionally silent. The exocytosis of quencher-loaded vesicles causes a fluorescence increase, an advantage for picking out signals against background noise. Extending this approach to neurons of different size

[illegible][illegible]

LATENT NO.		MIND DATE		ASSIGNMENT NO.		DATE	
N	44-38861	AL	1-1-1946	N	14-44-121491	1-1-1946	1-1-1946
N	44-38861	AL	1-1-1946				
RE: SA, H RE: AC, BE, CH, CY, DE, DF, EG, FI, FR, GR, HP, IR, IT, JO, KA, KL,							

Abstract: This review describes green fluorescent protein sensors, including their protein and encoding nucleotide sequences, which show reversible fluorescence changes of physiol. pH ranges for measuring the pH of a sample, and methods of use for measuring the pH of specific intracellular regions. The preferred fluorescent protein sensors are variants of the green fluorescent protein (GFP) from *Aequora victoria*. Also discussed are compns. and methods for measuring the pH of a specific region of a cell, such as the mitochondrial matrix or the Golgi lumen. Some sequence listings were not provided by the authors.

121. ANSWER TO OF 1: HARPLUS COPYRIGHT 2 0; ACC  
 ACCESSION NUMBER: 1999:014-75 HARPLUS  
 DOCUMENT NUMBER: 131:34574  
 TITLE: Tandem fluorescent protein  
 constructs and their preparation for enzyme assays  
 INVENTOR(S): Tsien, Roger Y.; Boix, Roger; Cubitt, Andrew  
 PATENT ASSIGNEE(S): The Regents of the University of California, USA;  
 Aurora Biosciences Corporation  
 SOURCE: U.S., 33 pp., Cont.-in-part of U.S. Ser. No. 894,177.  
 CODEN: YXXNAX  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY AND NUM. COUNT: 2  
 PATENT AND PUBL. NO:

PROPOSAL NUMBER	PROPOSED PROJECT	PROPOSED BUDGET	PROPOSED FUNDING SOURCE	STATUS
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81	81.1	81.1	81.1	81.1
82	82.1	82.1	82.1	82.1
83	83.1	83.1	83.1	83.1

THESE ARE UNCLASSIFIED REFERENCES AVAILABLE IN THIS REPORT. ALL OTHERS AVAILABLE IN THIS REPORT ARE UNCLASSIFIED.

FILE NUMBER: 100-436217 MEDLINE  
 AGENCY NUMBER: 100-436217 MEDLINE  
 DOCUMENT NUMBER: 100-436217 PubMed ID: 1141101  
 TITLE: Molecular permeation and receptor insertion within  
 green fluorescent proteins.  
 AUTHOR: Kuhl: G J; Zacharias: D A; Tsien: R Y  
 ORIGINATOR: Department of Pharmacology, University of California at San  
 Diego, La Jolla, CA 92037-0477, USA.  
 CONTRACT NUMBER:  
 SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE  
 UNITED STATES OF AMERICA, 1998 Vol 95 No 21, Pp 11411-11416.  
 Journal code: 7305876. ISSN: 0027-8424.  
 SUP. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 1998  
 ENTRY DATE: Entered STN: 19981101  
 Last Updated on STN: 19981101  
 Entered Medline: 19981101

As many new cloning, library and biotechnology have been revolutionized by the ability to label proteins genetically by fusion to the Acquire: green fluorescent protein (GFP). In previous fusions, the GFP has been treated as an individual entity, usually appended to the amino or carboxyl terminus of the host protein, occasionally inserted within the host sequence. The primary structure, three-dimensional structure and intricate posttranslational self-modification required for chromophore formation would suggest that major rearrangements or insertions within GFP would prevent fluorescence. However, we now show that several rearrangements of GFPs, in which the amino and carboxyl portions are interchanged and re-joined with a short spacer connecting the original termini, still become fluorescent. These circular permutations have altered pKa values and orientations of the chromophore with respect to the fusion partner. Furthermore, certain locations within GFP tolerate insertion of entire proteins, and conformational changes in the insert can have profound effects on the fluorescence. For example, insertions of alkali or a zinc finger remain in place: Tyr-145 of a yellow mutant enhanced yellow fluorescent protein (YEFP) GFP is essential for the insertion of a zinc finger whose fluorescence can be enhanced

Journal of Management Education 32(10) 1149-1162

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Journal of Management Education 33(10):1117-1122



LINE ANSWER 1 OF 14 MEDLINE  
ADDRESS NUMBER: 00-089601 MEDLINE  
DOCUMENT NUMBER: 00-0897 FORMED ID: 00-08911  
TITLE: An approach for reducing unwanted side-effects of cancer  
therapy proteins.  
AUTHOR: Avila Paul; Devenish Rodney J; Fraser Mark  
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Monash  
University, Clayton, Vic., Australia.  
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2004)  
Vol 319, Iss 1, P 21-24.  
Journal Code: MEDL. ISSN: 0045-2688.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal Article; JOURNAL ARTICLES  
LANGUAGE: English  
FILE FORMAT: Priority Journals  
ENTRY MONTH: 2004  
ENTRY DATE: Entered STN: 20021107  
Last Updated on STN: 20030116  
Entered Medline: 20030116

127 ANNEXES 1 OF 14 MEDLINE  
 A: ABSTRACT NUMBER: 127 MEDLINE  
 C: CITED NUMBER: 127  
 TITLE: Fluorescent labeling of protein iodellin.  
 AUTHOR: Brakov Arkady B; Vardolova Vladislav V; Chiravova Liliya  
 B; Polina Mariia B; Yankovskaya Yana I; Koryukov Vladimir  
 I; Lukyanov Sergey; Lukyanov Konstantin A  
 ORIGIN: 127  
 ORIGIN: 127

Submitted: 11 November 2014; Accepted: 10 February 2015



SOURCE: EMBL, Nucleotide-Sequence Data Bank, Moscow 117987, Russia.  
 JOURNAL: JOURNAL OF CELLULAR PHYSIOLOGY, Vol. 184, No. 1, pp. 1-10, 1999.  
 JOURNAL CODE: JCPH. ISSN: 0021-9594.  
 PUB. COUNTRY: England; United Kingdom  
 DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 1999  
 ENTRY DATE: Entered STN: 19990110  
 Last Updated STN: 19990110  
 Entered Medline: 19990110

ABSTRACT: Practical applications of **green fluorescent protein (GFP)** -like fluorescent proteins (FPs) from species of the class Anthozoa (sea anemones, corals and sea puffers) are strongly restricted owing to their **oligomeric nature**. Here we suggest a strategy to overcome this problem by the use of two covalently linked identical red FPs as **non-oligomerizing fusion tags**. We have applied this approach to the **dimeric red fluorescent protein** RFPd1 and have demonstrated superiority of the tandem tag in the in vivo labeling of the cytoskeletal structures and tiny organelles. In addition, a possibility of effective fluorescence resonance energy transfer (FRET) between enhanced yellow FP mutant 'EYFP' and tandem RFPd1 was demonstrated in a pulse assay.

KEYWORDS: CELL; MEDLINE  
 MEDLINE NUMBER: 19990110  
 DOCUMENT NUMBER: 19990110  
 TITLE: Intracellular localization of Herpes simplex virus type 1 thymidine kinase fused to different fluorescent proteins depends on choice of the reagent tag.  
 AUTHOR: Soling Ariane; Siam Andreas; Rainov Nikolai  
 CORPORATE SOURCE: Molecular Neurooncology Laboratory, Department of Neurosurgery, Martin-Luther-University Halle-Wittenberg, Heinrich-Luxemburg-Strasse 1, 06957, Halle, Germany..  
 ariane.soling@medizin.uni-halle.de  
 SOURCE: FEMS LETTERS, (2002 Sep 11) 229 (1-3) 153-8.  
 JOURNAL CODE: FLEP. ISSN: 0167-5194.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 2002  
 ENTRY DATE: Entered STN: 20020911  
 Last Updated STN: 20021218  
 Entered Medline: 20021218

ABSTRACT: Gene therapy employing the suicide gene product: activating system Herpes simplex virus type 1 thymidine kinase (HSV-TK)/ganciclovir (GCV) is efficient in killing malignant tumor cells. Labeling of the HSV-TK enzyme with fluorescent proteins makes possible the non-invasive imaging of transduction efficiency, enzyme localization and activity in cell culture and in animal models of human cancers. Here we report the expression of HSV-TK fused with different fluorescent proteins (EYFP, DsRed, IrfRed, dsRed1.6) and show that intracellular localization of the fusion product depends on the nature of the fluorescent tag despite the presence of several nuclear targeting signals within the enzyme itself. Coexpression of a different HSV-TK fused proteins with TK-EYFP or untagged HSV-TK did not allow proteins to enter the nucleus by inhibiting formation of red fluorescent protein oligomers. As enzyme localization may influence HSV-TK activity, this observation is of

potential for clinical use in therapy studies.

LAS ANKER # OF 24 MEDLINE  
 ACCESSION NUMBER: 144145 MEDLINE  
 DOCUMENT NUMBER: 144145 PubMed ID: 144145  
 TITLE: Measurement of changes in fluorescence resonance energy transfer between microaggregates-releasing hormone receptors in response to agonists.  
 AUTHOR: Cohen, Anna; Chan, F Michael  
 CORPORATE SOURCE: Johns Hopkins University, Center for Cell Biology and Molecular Physiology, Baltimore, MD, USA.  
 SOURCE: METHODS, (2012 Aug) 27(4): 304-32.  
 JOURNAL CODE: 0426-12. ISSN: 14-111.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE  
 LANGUAGE: English  
 FILE COMMENT: Priority Journals  
 ENTRY MONTH: 2012  
 ENTRY DATE: Entered STM: 12/12/12  
 Last Updated in STM: 12/12/12  
 Entered Medline: 12/12/12

**AB** **Oligomerization** of membrane- $\alpha$  and  $\beta$ -protein-coupled receptors has recently emerged as an important step in cellular signaling. Fluorescence resonance energy transfer (FRET) has emerged as a critical tool for demonstrating in vivo protein-protein interactions and receptor oligomerization. We have used oligomers of green fluorescent proteins (GFP) to investigate receptor dimerization in relation to receptor activation. Two pairs of FRET-compatible fluorescent proteins were used: Sapphire with Topaz, and enhanced green fluorescent protein (EGFP) with dsRed. Changes in the ratio between acceptor and donor fluorescence were measured after addition of buserelin, a GnRH agonist, and antide, a GnRH antagonist. For both pairs of fluorescent proteins, an increase in the ratio of acceptor to donor intensities was observed immediately after addition of buserelin, as would be predicted if FRET occurred due to the microaggregation of receptors conjugated with different fluorescent proteins. No change in FRET was observed in time for cells in medium or after addition of antide. The increase in FRET signal was not uniform throughout a cell.

LAS ANKER # OF 24 MEDLINE  
 ACCESSION NUMBER: 144145 MEDLINE  
 DOCUMENT NUMBER: 144145 PubMed ID: 144145  
 TITLE: Use of fluorescence resonance energy transfer to analyze oligomerization of  $\beta$ -protein-coupled receptors expressed in yeast.  
 AUTHOR: Cohen, Anna; Chan, F Michael  
 CORPORATE SOURCE: Department of Cell Biology and Physiology, Johns Hopkins University School of Medicine, 615 North Wolfe Avenue, St. Louis, MO 63110, USA.  
 SOURCE: METHODS, (2012 Aug) 27(4): 304-32.  
 JOURNAL CODE: 0426-12. ISSN: 14-111.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE  
 LANGUAGE: English  
 FILE COMMENT: Priority Journals  
 ENTRY MONTH: 2012  
 ENTRY DATE: Entered STM: 12/12/12  
 Last Updated in STM: 12/12/12  
 Entered Medline: 12/12/12

Entered Medline: 20021218

**AB** **Oligomerization** or dimerization of G-protein-coupled receptors (GPCRs) has emerged as an important theme in signal transduction. This concept has recently gained widespread interest due to the application of direct and noninvasive biophysical techniques such as fluorescence resonance energy transfer (FRET), which have shown unequivocally that several types of GPCR can form dimers or **oligomers** in living cells. Current challenges are to determine which GPCRs can self-associate and interact with other GPCRs, to define the molecular principles that govern these specific interactions, and to establish which aspects of GPCR function require **oligomerization**. Although these questions ultimately must be addressed by using GPCRs expressed in isolation in their native cell types, analysis of GPCR **oligomerization** in heterologous expression systems will be useful to survey which GPCRs can interact, to conduct structure-function studies, and to identify peptides or small molecules that disrupt GPCR **oligomerization** and function. Here, we describe methods employing scanning fluorimetry to detect FRET between GPCRs tagged with enhanced cyan and yellow fluorescent proteins (YFP and YFP) in living yeast cells. This approach provides a powerful means to analyze **oligomerization** of a variety of GPCRs that can be expressed in yeast, such as adrenergic, chemokine, Gs, muscarinic acetylcholine, vasopressin, opiate, and secretin receptors.

ACC. NUMBER: 20021218 MEDLINE  
 ABBREVIATION NUMBER: 20021218 MEDLINE  
 CURRENT NUMBER: 20021218 MEDLINE  
 TITLE: **Oligomerization** of GPCRs is required for the generation of a functional red fluorescent chromophore.  
 AUTHOR: Marchetti Andrea; Subramaniam Vinod; Levin Thomas M; Alberti Saverio  
 CORPORATE SOURCE: Laboratory of Experimental Oncology, Department of Cell Biology and Oncology, Istituto di Ricerche Farmacologiche Mario Negri-Consortio Mario Negri Sud, 66100, Chieti, Santa Maria Imbaro, Italy.  
 SOURCE: FEBS LETTERS, 2002 Aug 14; 525 (1-3): 13-9.  
 Journal code: 0168-9525, ISSN: 0014-5793.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE  
 LANGUAGE: English  
 FILE SEGMENT: Integrity Journals  
 ENTRY MONTH: 200209  
 ENTRY DATE: Ent-red STN: 20020917  
 Last Updated on STN: 20021218  
 Ent-red Medline: 20021218

**AB** The coral red fluorescent protein (DsRed) absorbs and emits light at much higher wavelengths than the structurally homologous **green** fluorescent protein, raising questions about the properties of its chromophore. We have analyzed the relationship between the aggregation state and fluorescence of native, c-histidine-tagged, or maltose-binding protein-fused DsRed. In all cases, newly synthesized DsRed molecules were largely monomeric and devoid of covalently closed chromophores. Maturation in vitro induces the expression of red **fluorescent** chromophores but only in **oligomeric** forms of the **protein**, whereas monomers are essentially devoid of fluorescence. NaOH-denatured samples demonstrated a generalized breakdown of the DsRed **oligomers** to monomers, which refolded after neutralization into weakly **green** fluorescent and still monomeric species. Red fluorescent chromophores were regenerated only upon **oligomerization**. These findings demonstrate that 'red'

[illegible]

While phages are linear and non-replicating they are **oligomers**, and appear that the smallest replicon has not been identified. A comparison of a linear, single-strand genome with a double-stranded genome is a comparison of two different types of molecules. **Oligomerization** may play a critical role in the replication of phages.

DOC. NUMBER: 1 F 114  
 APPROVAL NUMBER: 100-114  
 DOCUMENT NUMBER: 100-114  
 TITLE: Oligomerization of green  
 fluorescent protein in the secretory  
 pathway of and green cells.  
 AUTHOR: Paul R. W.; Lyons E. B.; K. Kondo M.; Hsiao J. A.; Paul J. W.  
 AFFILIATE: J. Molecular, Cellular and Developmental Biology,  
 University of Louisville, 101 South Preston Street,  
 Louisville, KY 40202, U.S.A.  
 JOURNAL NUMBER: 100-114  
 JOURNAL: BIOCHEMICAL JOURNAL, 1991, 274, 1-10.  
 JOURNAL DATE: 1991-01-01  
 JOURNAL VOLUME: 274  
 JOURNAL ISSUE: 1  
 JOURNAL PAGE: 1-10  
 JOURNAL TITLE: BIOCHEMICAL JOURNAL  
 JOURNAL TYPE: Journal; Article; JOURNAL ARTICLE  
 LANGUAGE: English  
 FILE NUMBER: 100-114  
 ENTRY NUMBER: 100-114  
 ENTRY DATE: 1991-01-01  
 ENTRY STN: 100-114  
 ENTRY STN: 100-114  
 ENTRY STN: 100-114

As Green et al. (1993) showed, GFP functioned extensively as a reporter protein to monitor cellular processes, including intracellular protein trafficking and secretion. In general, this system depends on GFP acting as a passive reporter protein. However, it was recently noted that GFP oligomerizes in the secretory pathway of endocrine cells. To characterize this oligomerization and its potential role in GFP transport, cytosolic and secretory forms of enhanced GFP (EGFP) were expressed in GH4C1 and AtT-2 endocrine cells. Biochemical analysis showed that cytosolic EGFP existed as a monomer, whereas secretory forms of EGFP formed disulphide-linked oligomers. EGFP contains two cysteine residues, Cys 48 and Cys 517, which multipley a role in this oligomerization. Site-directed mutagenesis of Cys 48 and Cys 517 showed that both cysteine residues were involved in disulphide interactions. Substitution of either cysteine residue resulted in a reduction or loss of oligomers, although dimers of the secretory form of EGFP remained. Mutation of these residues did not adversely affect the transference of EGFP. EGFP oligomers were stored in secretory granules and secreted by the regulated secretory pathway in endocrine AtT-2 cells. Similarly, the oligomeric form of EGFP was still secreted via the regulated secretory pathway, indicating that the high-order oligomers were not necessary for sorting in AtT-2 cells. These results suggest that the oligomerization of EGFP must be considered when the protein is used as a reporter molecule in the secretory pathway.

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101 ANSWER = P 194 MEDLINE
102 SYNONYM ANSWER: A 167847 MEDLINE
103 CURRENT NUMBER: M 161 MEDLINE
TITLE: Oligomerization of the human amyloid  $\beta$ -protein  

       1-42: fluorescent protein-tagged

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$\frac{1}{2} \leq \frac{1}{2} \leq \frac{1}{2}$

ABSTRACT: TSHR reveals post-translational complexes.  
 AUTHOR: Lavi E; Haves E; Lavi E F  
 CORPORATE SOURCE: Division of Endocrinology, Diabetes and Bone Diseases,  
 Mount Sinai School of Medicine, New York, New York  
 10029-3074, USA. Email: lavi@msm.mssm.edu  
 CONTACT NUMBER: 212 6594400-1111  
 DE-1004 NIIIF  
 DE-1011 NIIIF  
 DE-1044 NIIIF

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, 276(11):4011-4014,  
 2001-11-16.  
 Journal Code: JBC11116. ISSN: 0021-9758.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE  
 LANGUAGE: English  
 FILE COMMENT: Priority Journals  
 ENTRY MONTH: 2001  
 ENTRY DATE: Entered STN: 20011116  
 Last Updated on STN: 20011116  
 Entered Medline: 20011116

AB: To examine thyrotropin (TSH) receptor homophilic interactions we used the human TSH receptor (hTSHR) carboxyl terminus as **green** fluorescent protein (GFP) and the corresponding chimeric DNA was expressed in Chinese hamster ovary cells. Fluorescent TSH receptors on the plasma membrane were functional as assessed by TSH-induced cAMP synthesis. The clustering of TSHR, as well as hTSHR autoantibodies, induced TSH- and dose-dependent receptor coupling. Fluorescence resonance energy transfer between receptors differentially tagged with **GFP** variants (YFP and YFP) provided evidence for the close proximity of individual receptor molecules. This was consistent with previous studies demonstrating the presence of TSHR dimers and oligomers in thyroid tissue. Co-immunoprecipitation of **GFP**-tagged and Myc-tagged receptor complexes was performed using doubly transfected cells with Myc antibody. Western blotting of the immunoprecipitated complex revealed the absence of noncleaved TSH holoreceptors. This further suggested that cleavage of the holoreceptor into its two-subunit structure, comprising disulfide-linked TSHR-alpha and TSHR-beta subunits, was required for the formation of TSHR dimers and higher order complexes.

DE-1004 NIIIF MEDLINE  
 ACCESSION NUMBER: 2001219271 MEDLINE  
 DOCUMENT NUMBER: 21265944 PubMed ID: 1119274  
 TITLE: Self-assembly and binding of a sorting nexin to sorting endosomes.  
 AUTHOR: Korten R C; Eddington A L; Chowdhury P; Smith R L; Davidson A L; Shank B E  
 CORPORATE SOURCE: Department of Physiology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205-7500, USA.  
 KortenRichard@exchange.uams.edu  
 SOURCE: JOURNAL OF CELL SCIENCE, (2001 May) 114(Pt 5):1174-1180.  
 Journal Code: JCS2400. ISSN: 0021-9792.  
 PUB. COUNTRY: England; United Kingdom  
 DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE  
 LANGUAGE: English  
 FILE COMMENT: Priority Journals  
 ENTRY MONTH: 2001  
 ENTRY DATE: Entered STN: 20011116  
 Last Updated on STN: 20011116  
 Entered Medline: 20011116

AP The rate of endoplasmic reticulum protein and luminal retention is determined by a molecular processing system in a still elusive way. Endoplasmic retention is a mechanism that traps specific proteins within this compartment, and thereby prevents their recycling. We report that a certain form of SNX1, a transmembrane protein, is a protein that is retained in the ER in vitro and in vivo, and has this property in common with the yeast protein Yop1p. A comparison of SNX1 expressed in bacterial and mammalian systems, and analysed by size-exclusion chromatography, indicated that in yeast SNX1 tetramers are part of a larger complex with luminal proteins. An endoplasmic retention mechanism would require that SNX1 should have a signal sequence, yet the complexes that we analysed were largely soluble and little SNX1 was found in pellet fractions. Using **green fluorescent protein fusions**, and cytic compartment markers and recovery after pH titration, we found that there is an equilibrium between late cytoplasmic and early sorting endoplasmic protein **green fluorescent protein-SNX1**. Fluorescence resonance energy transfer indicated that spectral variants of **green fluorescent protein-SNX1** were oligomeric in vivo. In cell extracts, these **green fluorescent protein-SNX1 oligomers** corresponded to tetramers and larger complexes of **green fluorescent protein-SNX1**. Using wide-angle X-ray diffraction, we observed small vesicle binding and fusion with a large **green fluorescent protein-SNX1** coated vesicle, which are features consistent with their role as sorting endoplasmic way.

<http://www.sciencedirect.com/science/article/pii/S0006291X00000000>

LAST ANSWER: 10/14/2000 MEDLINE  
 ABSTRACT NUMBER: 1000000000 MEDLINE  
 DOCUMENT NUMBER: 1000000000 MEDLINE  
 TITLE: RNA chemistry, mutagenesis, and oligomerization of Isk-d, a red fluorescent protein from coral.  
 AUTHOR: Baird G S; Zacharias D A; Tsien R Y  
 CORPORATE SOURCE: Department of Pharmacology, University of California, San Diego, La Jolla, CA 92037, USA.  
 CONTRACT NUMBER: N0100000000 (NINDS)  
 SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2000) OCT 24, 97 (22), 11964-9. Journal code: 0006-291X, ISSN: 0006-291X.  
 COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 10/14  
 ENTRY DATE: Entered STN: 1000000000  
 Last Updated on STN: 2/10/2002  
 Entered Medline: 20001115

AP Isk-d is a recently cloned 26-kDa fluorescent protein responsible for the red coloration around the oral disk of a coral of the *Discozoma* genus. Isk-d has attracted tremendous interest as a potential expression tracer and fusion partner that would be complementary to the numerous **green fluorescent protein** from *Aequorea*, but very little is known of the biochemistry of Isk-d. We now show that Isk-d has a much higher extinction coefficient and quantum yield than previously reported, plus excellent resistance to pH extremes and photobleaching. In addition, the Isk-d emission maximum can be further shifted to 650 nm by mutation of lys-100 to Met. However, Isk-d has major drawbacks, such as strong **oligomerization** and slow maturation. Analytical ultracentrifugation proves Isk-d to be an obligate tetramer in vitro, and

The two main and most striking features of the polymerization are: (1) the
 rapid **oligomerization** in the initial stage, (2) the gradual
 polymerization which follows. The color of the polymer changes from
 green to yellow to orange to red. The color change is due to the
 formation of the polymer. The color change is due to the formation of the
 polymer. The color change is due to the formation of the polymer.

cells which use RNA as their genetic material within viral particles and DNA provirus as their genetic material within cells. The rate of recombination during reverse transcription between two identical sequences within the same RNA molecule is very high. In this study, we have developed a sensitive system to study recombination occurring within the proviral sequence. This system includes a murine Moloney leukemia virus vector which contains a neomycin resistance gene (neo<sup>r</sup>) and two mutated green fluorescent protein genes (gfp) in tandem positions. The 3' end of the first gfp and the 5' end of the second gfp gene are both mutated, so that neither of these two gfp genes is functional. However, if recombination occurs between the two gfp genes it will create a functional gfp protein. Cells containing such a functional recombinant gfp appear green under fluorescence microcopy. The rate of recombination between the two gfp sequences during a single round of replication is as high as 61%. Green cells appear during proliferation of a clonal cleared-cell population and allow a small portion of these recombinations between sequences of proviral DNA to be detected. The frequency of recombination at the proviral DNA level is about 1 - 5 events/cell/division, which is very low compared with the frequency of recombination (61%) caused by reverse transcriptase and/or RNA polymerase II.

ID: ANSWER 11 F 11 MEDLINE  
 ABSTRACT NUMBER: 100000000 MEDLINE  
 DOCUMENT NUMBER: 100000000 PubMed ID: 100000000  
 TITLE: Effect of N-terminal alpha-helix formation on the  
 localization and intracellular targeting of  
 a heterodimeric amino transferase.  
 AUTH: [unreadable] [unreadable] [unreadable]  
 J. Biol. Chem. 269: 10000-10000 (1994)

PubMed ID: 9149559

Department of Biology, University College London, Gower Street, London W1E 6BT, United Kingdom.  
JOURNAL OF BIOLOGICAL CHEMISTRY, 1997 Vol 272, Pt 1, 174-181.  
Journal Code: JBCB111R. ISSN: 0021-9975.  
Pub. Country: United States  
Document Type: Journal; Article; JOURNAL ARTICLE  
Language: English  
File Format: Bibliography  
Entry Count: 1 entry  
Entry Date: Entered EMBL: 1997-01-17  
Last Updated on EMBL: 1997-01-17  
Entered Medline: 1997-01-17

AB The mislocalized peroxisome- $\alpha$ -mitochondrion mistargeting of dominantly xylate aminotransferase (AGT) in the hereditary disease primary hyperoxaluria type 1 is caused by the combined presence of a common Pro11  $\rightarrow$  Leu polymorphism and a disease-specific Gly17  $\rightarrow$  Arg mutation. The Pro11  $\rightarrow$  Leu replacement generates a functionally weak N-terminal mitochondrial targeting sequence (MTS), the efficiency of which is increased by the additional presence of the Gly17  $\rightarrow$  Arg replacement. AGT dimerization is inhibited in the combined presence of both replacements, but not when each is present separately. In this paper we have attempted to identify the structural determinants of AGT dimerization and mitochondrial mistargeting. Unlike most MTSs, the polymorphic MTS of AGT has little tendency to adopt an alpha-helical conformation in vitro. Nevertheless, it is able to target efficiently a non-membrane **green fluorescent (GFP) fusion protein**, but not **dimeric AGT**, to mitochondria in transfected COS-1 cells. Increasing the propensity of this MTS to fold into an alpha-helix, by making a double Pro11  $\rightarrow$  Leu + Pro10  $\rightarrow$  Leu replacement, enabled it to target both **GFP** and AGT efficiently to mitochondria. The double Pro11  $\rightarrow$  Leu + Pro10  $\rightarrow$  Leu replacement retarded AGT dimerization in vitro as did the disease-causing double Pro11  $\rightarrow$  Leu + Gly170  $\rightarrow$  Arg replacement. These data suggest that N-terminal alpha-helix formation is more important for maintaining AGT in a conformation (i. e. monomeric) compatible with mitochondrial import than it is for the provision of mitochondrial targeting information. The parallel effects of the Pro10  $\rightarrow$  Leu and Gly17  $\rightarrow$  Arg replacements on the dimerization and intracellular targeting of polymorphic AGT (containing the Pro11  $\rightarrow$  Leu replacement) raise the possibility that they might achieve their effects by the same mechanism.

KEY ANSWER ID: 9149559 MEDLINE  
ACCESSION NUMBER: 97446032 MEDLINE  
DOCUMENT NUMBER: 97446032 PubMed ID: 9149559  
TITLE: **Oligomerization of expanded-polyglutamine domain fluorescent fusion proteins in cultured mammalian cells.**  
AUTHOR: Chivers G; Burke J K; Miller J E; Hester J; Tendi J; Jones A G; Strittmatter W J  
CORRESPONDING AUTHOR: Department of Medicine (Neurology), Duke University Medical Center, Durham, North Carolina 27710, USA.  
CONTRACT NUMBER: 5-1-97-CA-14236-04 (NIH)  
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 1997 Vol 231, Pt 2, 122-125.  
Journal Code: JBCB111R. ISSN: 0006-291X.  
Pub. Country: United States  
Document Type: Journal; Article; JOURNAL ARTICLE  
Language: English

Search completed by David Schofield 1997-01-17



Exp. Neurol., 1997

FILE NUMBER: Priority Journal  
ENTRY NUMBER: 14771  
ENTRY DATE: August 1997: 14771-14  
Last Modified: 14771-14  
Entered: 14771-14

AB Cerebral neurodegenerative diseases, including Huntington's disease, result from the expansion of a CAG repeat in the disease gene to produce a domain of more than 36 glutamines in the expressed protein. The mechanism by which expansion of this polyglutamine domain causes disease is unknown. Recent studies have indicated **oligomerization** of polyglutamine domain proteins in mammalian neurons. To study **oligomerization** of polyglutamine proteins and to identify heterodimeric protein interactions, varying length polyglutamine-**green** fluorescent protein fusion proteins were expressed in cultured cells. The 16- and 25-glutamine fusion proteins were stable and length distributed diffusely throughout the cytoplasm. In contrast, 36- and 41-glutamine fusion proteins produced length forms: fibrillar arrays resembling those previously observed in neurons in Huntington's disease and in a transgenic mouse model. These aggregates were intranuclear and intracytoplasmic. Intracytoplasmic aggregates were surrounded by collapsed intermediate filaments. The intermediate filament protein vimentin co-immunoprecipitates with expanded polyglutamine fusion proteins. This cellular model will expedite investigations into **oligomerization** of polyglutamine proteins and their interactions with other proteins.  
Copyright 1997 Academic Press.

DOI: 10.1006/0014-7167(97)90014-0

ACCESSION NUMBER: 14771-14

DOCUMENT NUMBER: 14771-14

TITLE: Systemically delivered antisense oligomers upregulate gene expression in mouse tissues

AUTHOR(S): Nazani, Peter; Semignani, Federica; Kang, Shin-Hong; Maier, Martin A.; Manoharan, Muthiah; Persmark, Magnus; Bortner, Donna; Kole, Ryszard

ORGANIZATION: Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, 27599, USA

SOURCE: Nature Biotechnology 15(12), 1228-1233

CODEN: NABIF9; ISSN: 1087-0156

PUBLISHER: Nature Publishing Group

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Systemically injected 2'-O-methoxyethyl 2'-O-MOE)-phosphorothioate and RNA-4K oligomers (peptide **nucleic** acid with four lysines linked at the 3' terminus) exhibited sequence-specific antisense activity in a number of mouse organs. Morpholino oligomers were less effective, whereas RNA oligomers with only the lysine (RNA-1K) were completely inactive. The latter result indicates that the four-lysine tail is essential for the antisense activity of RNA oligomers in vivo. These results were obtained in a transgenic mouse model designed as a positive readout test for activity, delivery, and distribution of antisense oligomers. In this model, the expressed gene EGFP-634 encoding enhanced **green** fluorescence protein (EGFP) is interrupted by an aberrantly spliced mutated intron of the human beta-actin gene. Aberrant splicing of this intron prevented expression of EGFP-634 in all tissues, whereas in tissues and organs that express a splice site-targeted antisense oligomer, correct splicing was restored and EGFP-634 expression upregulated. The sequence-specific activity of RNA-4K and the 2'-O-MOE oligomers to upregulate EGFP-634 provides strong evidence that systemically delivered, chem. modified

[illegible]

Table 1. *Continued*

[illegible]

PATENT N.°				FILING DATE				APPLICATION N.°				DATE			
N.°				FILING DATE				N.°				FILING DATE			
01:	AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC, CD, CE, CF, CG, CH, CI, CJ, CK, CL, CM, CN, CO, CP, CQ, CR, CS, CT, CU, CV, CW, CX, CY, CZ, DA, DB, DC, DD, DE, DF, DG, DH, DI, DJ, DK, DL, DM, DN, DO, DP, DQ, DR, DS, DT, DU, DV, DW, DX, DY, EA, EB, EC, ED, EE, EF, EG, EH, EI, EJ, EK, EL, EM, EN, EO, EP, EQ, ER, ES, ET, EU, EV, EW, EX, EY, EZ, FA, FB, FC, FD, FE, FF, FG, FH, FI, FJ, FK, FL, FM, FN, FO, FP, FQ, FR, FS, FT, FU, FV, FW, FX, FY, GA, GB, GC, GD, GE, GF, GH, GI, GJ, GK, GL, GM, GN, GO, GP, GQ, GR, GS, GT, GU, GV, GW, GX, GY, HA, HB, HC, HD, HE, HF, HG, HH, HI, HJ, HK, HL, HM, HN, HO, HP, HQ, HR, HS, HT, HU, HV, HW, HX, HY, IA, IB, IC, ID, IE, IF, IG, IH, II, IJ, IK, IL, IM, IN, IO, IP, IQ, IR, IS, IT, IU, IV, IW, IX, IY, JA, JB, JC, JD, JE, JF, JG, JH, JI, JJ, JK, JL, JM, JN, JO, JP, JQ, JR, JS, JT, JU, JV, JW, JX, JY, KA, KB, KC, KD, KE, KF, KG, KH, KI, KJ, KK, KL, KM, KN, KO, KP, KQ, KR, KS, KT, KU, KV, KW, KY, LA, LB, LC, LD, LE, LF, LG, LH, LI, LJ, LK, LL, LM, LN, LO, LP, LQ, LR, LS, LT, LU, LV, LW, LX, LY, MA, MB, MC, MD, ME, MF, MG, MH, MI, MJ, MK, ML, MM, MN, MO, MP, MQ, MR, MS, MT, MU, MV, MW, MX, MY, NA, NB, NC, ND, NE, NF, NG, NH, NI, NJ, NK, NL, NM, NN, NO, NP, NQ, NR, NS, NT, NU, NV, NW, NX, NY, OA, OB, OC, OD, OE, OF, OG, OH, OI, OJ, OK, OL, OM, ON, OO, OP, OQ, OR, OS, OT, OU, OV, OW, OX, OY, PA, PB, PC, PD, PE, PF, PG, PH, PI, PJ, PK, PL, PM, PN, PO, PP, PQ, PR, PS, PT, PU, PV, PW, PX, PY, QA, QB, QC, QD, QE, QF, QG, QH, QI, QJ, QK, QL, QM, QN, QO, QP, QQ, QR, QS, QT, QU, QV, QW, QX, QY, RA, RB, RC, RD, RE, RF, RG, RH, RI, RJ, RK, RL, RM, RN, RO, RP, RQ, RR, RS, RT, RU, RV, RW, RX, RY, SA, SB, SC, SD, SE, SF, SG, SH, SI, SJ, SK, SL, SM, SN, SO, SP, SQ, SR, SS, ST, SU, SV, SW, SX, SY, TA, TB, TC, TD, TE, TF, TG, TH, TI, TJ, TK, TL, TM, TN, TO, TP, TQ, TR, TS, TU, TV, TW, TX, TY, UA, UB, UC, UD, UE, UF, UG, UH, UI, UJ, UK, UL, UM, UN, UO, UP, UQ, UR, US, UT, UV, UW, UX, UY, VA, VB, VC, VD, VE, VF, VG, VH, VI, VJ, VK, VL, VM, VN, VO, VP, VQ, VR, VS, VT, VU, VW, VX, VY, WA, WB, WC, WD, WE, WF, WG, WH, WI, WJ, WK, WL, WM, WN, WO, WP, WQ, WR, WS, WT, WU, WV, WW, WX, WY, XA, XB, XC, XD, XE, XF, XG, XH, XI, XJ, XK, XL, XM, XN, XO, XP, XQ, XR, XS, XT, XU, XV, XW, XX, XY, YA, YB, YC, YD, YE, YF, YG, YH, YI, YJ, YK, YL, YM, YN, YO, YP, YQ, YR, YS, YT, YU, YV, YW, YX, YY, ZA, ZB, ZC, ZD, ZE, ZF, ZG, ZH, ZI, ZJ, ZK, ZL, ZM, ZN, ZO, ZP, ZQ, ZR, ZS, ZT, ZU, ZV, ZW, ZX, ZY														
02:	01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99														
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08:	01, 02, 03, 04, 05, 06, 07, 08, 09,														

AB The invention relates to proteins or polypeptides that comprise intramol-  
dimers of fluorescent protein monomers. More  
specifically, the invention relates to recombinant polypeptides comprising  
a monomer of a fluorescent polypeptide, a linker peptide, and a second  
monomer of that fluorescent polypeptide, where the monomers form an  
intramol. dimer. The invention also relates to nucleic acids  
encoding intramol. Dimer Fluorescent Proteins  
IDFPs and vectors comprising such a nucleic acids. The  
invention further relates to methods of making IDFPs and methods of using  
them. IDFPs are useful in any application suited for fluorescent proteins  
and are particularly useful in applications in which more than one  
fluorescent protein sharing complementary  
dimerization interfaces is present in the same mixt. It is  
convenient in the same mixt, because IDFPs do not form heterodimers.

[illegible]

PRIORITY MAILING INDEX: RE 10-74 2001 A

AB The present invention relates to **nucleic acids** encoding S11  
"selected" interacting domain polypeptides; minimal fragments of hepatitis  
C virus proteins responsible for specific interactions with proteins of  
the virus. These peptides and their interactions may be targets for  
prevention and treatment of infection of hepatitis C virus. The invention  
also includes vectors comprising a **nucleic acid** encoding a S11  
polypeptide as well as host cells transformed with such vectors. The  
invention is also directed to yeast or bacterial two-hybrid methods which  
make use of the **nucleic acids** encoding a S11 polypeptide  
selected from a pathogenic strain of the hepatitis C virus as well as to  
methods for selecting moles, which inhibit the binding between a S11  
polypeptide and a polypeptide which specifically binds thereto.  
Protein-protein interactions of S11 polypeptides may be detected by  
**covalent or non-covalent** attachment of  
**fluorescent proteins**, labeled antibodies or enzymes with  
catalytic activity to one S11 polypeptide (marker protein) and  
subsequently contacting the marker protein with a plurality of other  
proteins like S11 polypeptides. The S11 polypeptides may also be  
covalently linked to a spacer, which may also be covalently bound to a  
substrate to immobilize the S11 polypeptides. For example, the S11  
polypeptide may be attached to biotin when the substrate is streptavidin.  
Changes in optical properties of the substrate, following binding by S11  
polypeptides are detected. S11 polypeptides can be used in vaccines for  
prevention and treatment of hepatitis C virus in animals or humans.

ABSTRACT NUMBER: E 1:49-41- E VALLEY  
 DOCUMENT NUMBER: 148:02764-  
 TITLE: Single molecule imaging of **green** fluorescent proteins in living cells: E-cadherin forms **oligomers** on the free cell surface  
 AUTHOR(S): Iino, Ryota; Koyama, Ikuko; Kusumi, Akihito  
 CORPORATE SOURCE: Kusumi Membrane Organizer Project, Exploratory Research for Advanced Technology Organization, Japan Science and Technology Corporation, Nagoya, 4-1-1, Japan  
 SUBJECT: Biophysical Journal, 2011, 103(1), 1-14



Page 1 of 1

transporter and of the rat 5HTA transporter 1  
visualized by fluorescence resonance energy transfer  
microscopy in living cells

AUTHOR(S):  
Jurek, Thomas A.; Schulze, Ines; Mariani, Oliver;  
Friedmann, Michael; Singer, Ernst A.; Jurek, Harald  
B.

ORGANATE SOURCE:  
Department of Vascular Biology and Transcels  
Research, University of Vienna Medical School, Vienna,  
A-1040, Austria

JOURNAL:  
Journal of Biological Chemistry 277, 10000,  
1999-10-01

CITEN: JBCBA; ISSN: 0021-9758

PUBLISHER:  
American Society for Biochemistry and Molecular  
Biology

DOCUMENT TYPE:  
Journal

LANGUAGE:  
English

ABSTRACT: Previous studies indicate that the serotonin transporter can form  
oligomers. We investigated whether the human serotonin  
transporter (hSERT) can be visualized as an oligomer in the  
plasma membrane of intact cells. For this purpose, we generated fusion  
proteins of hSERT and spectral variants of the green fluorescent  
protein (GFP) and yellow fluorescent proteins, YFP and YFP, respectively. When  
expressed in human embryonic kidney 293 cells, the resulting fusion  
proteins GFP-hSERT and YFP-hSERT were efficiently inserted into the  
plasma membrane and were functionally indistinguishable from wild-type  
hSERT. Oligomers were visualized by fluorescence resonance  
energy transfer microscopy in living cells using two complementary  
methods, i.e., confocal imaging and acceptor photobleaching. Interestingly,  
oligomerization was not confined to hSERT; fluorescence resonance  
energy transfer was also seen between YFP- and YFP-labeled rat  
5-hydroxytryptamine transporter. The bulk of serotonin transporters  
was recovered as high mol. wt. complexes upon gel filtration in detergent  
solution. In contrast, the monomers of GFP-hSERT and YFP-hSERT were  
essentially undetectable. This indicates that the homo-oligomeric  
form is the favored state of hSERT in living cells, which is not  
significantly affected by coincubation with transporter substrates or  
blockers. Based on our observations, we conclude that constitutive  
oligomer formation might be a general property of  
Na<sup>+</sup>/K<sup>+</sup>-dependent neurotransmitter transporters.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2: ANSWER 2: OF 24 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:079549 HCAPLUS

COMMENT NUMBER: 130:35223

TITLE:  
FROM: proximity imaging of green fluorescent  
protein-tagged polypeptides

AUTHOR(S):  
De Angelis, Ilia A.; Klesse, John; Fendelman, Boris  
V.; Rothman, James E.

ORGANATE SOURCE:  
Cellular Biochemistry and Biophysics Program, Memorial  
Sloan-Kettering Cancer Center, New York, NY, 10021,  
USA

JOURNAL:  
Proceedings of the National Academy of Sciences of the  
United States of America 96, 12, 12012-12016  
CITEN: PNAS; ISSN: 0027-8124

PUBLISHER:  
National Academy of Sciences

DOCUMENT TYPE:  
Journal

LANGUAGE:  
English

ABSTRACT: We report a serendipitous discovery that extends the impressive catalog of

The first feature is governed by **green** and dependent on both  
**GFP** and the matrix. When the GFP is in the matrix, the  
 proximity, measured in the relative intensities of **green**  
 fluorescence emitted by an excited state of GFP, is small. These  
 spectral changes provide a sensitive radiometric index of the extent of  
 protein-matrix contact that can be exploited to quant. image of **oligomerization** : clustering processes of GFP-tagged  
 proteins in vivo. The method, which we term proximity imaging (PIIM),  
 implements a sensitive resonance energy transfer between GFP  
 and a donor protein and a GFP acceptor, a process that  
 is sensitive to proximity relative to GFP-tagged proteins. However,  
 unlike FRET, where the donor energy transfer, which is a spectral  
 interaction, PIIM depends on direct contact between the GFP  
 proteins, which can lead to structural perturbations and consequent  
 spectral changes within a module. Moreover, the precise spatial  
 arrangement of the GFP molecules within a given oligomer affects the  
 magnitude and direction of the spectral change. We have used PIIM to  
 detect FRET-induced dimerization of GFP fused to FRET-binding  
 protein and clustering of glycosylphosphatidylinositol-anchored  
 GFP in cell membranes.

THERE ARE NO OTHER REFERENCES AVAILABLE FOR THIS  
RECORD. ALL STATISTICS AVAILABLE IN THE RECORD FORMS

[illegible]

1. DATE OF BIRTH: 1950-01-01  
 2. DATE OF DEATH: 1950-01-01

AB Background: Rhodopsin knockout mice (rho-/-) lack outer segments, and their rod outer segment is nearly complete by post-natal day 14 (Hewes et al., 1997, JNAB, 74: 73-74). This study aimed to deliver rhodopsin to these animals via an adenovectorial virus (AdV2), and assess potential rescue efforts. Methods: An AdV2, expressing the murine rhodopsin cDNA in tandem with the marker enhanced green fluorescent protein was prepared. Rho-/- mice (post-natal days 8-9) subsequently received the virus subretinally (single eye) and were maintained for 12 weeks. Contralateral eyes served as untreated controls. Five, 8 and 12 weeks post-injection, eyes were enucleated, fixed, cryoprotected and assessed histologically. Samples were analysed for rhodopsin (primary antibody, rho4B2), and EGFP expression, as well as the presence of photoreceptor rescue, using fluorescent microcopy. Results: Five weeks post-injection, EGFP expression localized to photoreceptor cell bodies and inner segments. Immunohistochemistry revealed strong rhodopsin labelling localized to the tips of the inner segments. A similar expression pattern was also evident at 8 weeks post-injection. At this time there also appeared to be local rescue of photoreceptor surrounding the site of injection; however, there were no changes in the outer segment population, and the rescue effort was not

maintained by 12 weeks of live treatment eyes demonstrated a lack of control, and integrin and EGFP expression was no longer evident. Following AMH-mediated delivery of integrin to the - - mice also not appear to resolve the integrin phenotype. Results may be dependent on the time of delivery. The level of integrin expression and a mimic that of the normal embryonic state as well as the rate of onset of AMH-mediated integrin expression. Studies are underway to define whether AMH can type and to deliver the integrin in the , in order to maximize potential rescue effects.

LE: ANSWER 1000000000 BIOSIS COPYRIGHT 2000 BIOLOGICAL ABSTRACTS INC.  
 ABSTRACT NUMBER: 1000000000 BIOSIS  
 DOCUMENT NUMBER: EMBE0011000000  
 TITLE: Fluorescence anisotropy decay microscopy in living cells to measure monomer-dimer transition of GFP-tagged proteins.  
 AUTHOR(S): Gauthier, L.; Tranter, M.; Curieux, C.; Cappel, L.; Bouch, R. P.; Nicolas, L.-C.; Benoit, R.; Cappel-Moisan, M. J.  
 CORPORATE SOURCE: U. Institut Jacques Monod, UMR 7061, 2 Place Jussieu-Tour 4, 75251 Paris Cedex 05, France; e-mail: cappel@ijm.jussieu.fr  
 SOURCE: Biophysical Journal, June, Vol. 81, No. 6, pp. 2800-2808, 2001.  
 ISSN: 0006-3428.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English

AB Fluorescence anisotropy decay microscopy was used to determine, in individual living cells, the spatial monomer-dimer distribution of proteins, exemplified by herpes simplex virus thymidine kinase (TK fused to green fluorescent protein (GFP)). Accordingly, the fluorescence anisotropy dynamics of two fusion proteins (TK2GFP and TK366GFP) was recorded in the confocal mode by ultra-sensitive time-correlated single-photon counting. This provided a measurement of the rotational time of these proteins, which, by comparing with GFP, allowed the determination of their oligomeric state in both the cytoplasm and the nucleus. It also revealed energy homo-transfer within aggregates that TK366GFP progressively formed. Using a symmetric dimer model, structural parameters were estimated: the mutual orientation of the transition dipoles of the two GFP chromophores, calculated from the residual anisotropy, was 44.6-1.0 degree, and the upper intermolecular limit between the two fluorescent tags, calculated from the energy transfer rate, was 20 ANG. Acquisition of the fluorescence steady-state intensity, lifetime, and anisotropy decay in the same cells, at different times after transfection, indicated that TK366GFP was initially in a monomeric state and then formed dimers that grew into aggregates. Fluorescence time-resolved fluorescence anisotropy microscopy opens a promising avenue for obtaining structural information on proteins in individual living cells, even when expressed at levels as very low.

LE: ANSWER 1000000000 BIOSIS COPYRIGHT 2000 BIOLOGICAL ABSTRACTS INC.  
 ABSTRACT NUMBER: 1000000000 BIOSIS  
 DOCUMENT NUMBER: EMBE0006000000  
 TITLE: A novel nuclear receptor heterodimerization pathway mediated by orphan receptors TR2 and TR4.  
 AUTHOR(S): Lee, Chih-Hao; Chinpaisal, Chantai; Wei, Li-Na et al.  
 CORPORATE SOURCE: U. Dep. Pharmacol., Univ. Minnesota, 6-347 Millard Hall, 435 Delaware St. S.E., MN 55455 USA  
 SOURCE: Journal of Biological Chemistry, 276(11), 10000-10006, 2001, Vol. 276, No. 11, pp. 10000-10006.

Trial	Control (n=10)	MCI (n=10)	AD (n=10)
1	85	75	65
2	82	72	62
3	78	68	58
4	76	66	56
5	75	65	55

[illegible]

AB A single defined interaction pathway involving ligand receptors Tg1 and Tg2 is sufficient. Tg1 and Tg2 preferentially interact with each other as well as with DNA elements containing a short repeat (TR). The in vitro interaction between Tg1 and Tg2 is demonstrated by the yeast two-hybrid system, two-hybrid interaction assays, the pull-down assay, and the co-localization assay. The in vivo interaction is demonstrated by colocalization of a Tg1 and Tg2 fusion protein with a green fluorescent protein. The dimerization is mediated by the ligand binding domain, and the three leucine residues in domain I of Tg1 are critical for this interaction. In addition, coexpression of these two genes is essential for the growth of the cells, and a Tg1-containing cell line that expresses either receptor alone. In the developing muscle, Tg1 and Tg2 are coexpressed in the same population of myoblasts and exhibit a parallel pattern of expression during development. The preferential heterodimerization between Tg1 and Tg2 and their coexistence in specific cell line suggest a physiological role of the Tg1-Tg2 interaction in muscle development.

ANALYSIS OF THE EFFECTS OF THE 1997-1998 EL NIÑO ON THE RAINFALL AND TEMPERATURE PATTERNS IN THE TROPICAL AMERICAS

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TITLE: 19901001001 and 19901001002 : GFP-19901001001  
 19901001001: 19901001001 and 19901001002 : 19901001001

[illegible]

FOR FURTHER INFO: Dr. R.A. Decker, Molecular Pharmacology, AstraZeneca  
Laboratories, 1110 Torreyana Road, San Diego, CA 92121,  
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INSTR: 10-7-2571 CODEN: JRISEJ

1. **CONCLUSIONS**

[illegible]

<p> <b>INDEX</b>  <b>BY SUBJECT</b>  <b>AND</b>  <b>BY AUTHOR</b> </p>	<p> <b>1-100</b>  <b>101-200</b>  <b>201-300</b>  <b>301-400</b>  <b>401-500</b>  <b>501-600</b>  <b>601-700</b>  <b>701-800</b>  <b>801-900</b>  <b>901-1000</b>  <b>1001-1100</b>  <b>1101-1200</b>  <b>1201-1300</b>  <b>1301-1400</b>  <b>1401-1500</b>  <b>1501-1600</b>  <b>1601-1700</b>  <b>1701-1800</b>  <b>1801-1900</b>  <b>1901-2000</b>  <b>2001-2100</b>  <b>2101-2200</b>  <b>2201-2300</b>  <b>2301-2400</b>  <b>2401-2500</b>  <b>2501-2600</b>  <b>2601-2700</b>  <b>2701-2800</b>  <b>2801-2900</b>  <b>2901-3000</b>  <b>3001-3100</b>  <b>3101-3200</b>  <b>3201-3300</b>  <b>3301-3400</b>  <b>3401-3500</b>  <b>3501-3600</b>  <b>3601-3700</b>  <b>3701-3800</b>  <b>3801-3900</b>  <b>3901-4000</b>  <b>4001-4100</b>  <b>4101-4200</b>  <b>4201-4300</b>  <b>4301-4400</b>  <b>4401-4500</b>  <b>4501-4600</b>  <b>4601-4700</b>  <b>4701-4800</b>  <b>4801-4900</b>  <b>4901-5000</b>  <b>5001-5100</b>  <b>5101-5200</b>  <b>5201-5300</b>  <b>5301-5400</b>  <b>5401-5500</b>  <b>5501-5600</b>  <b>5601-5700</b>  <b>5701-5800</b>  <b>5801-5900</b>  <b>5901-6000</b>  <b>6001-6100</b>  <b>6101-6200</b>  <b>6201-6300</b>  <b>6301-6400</b>  <b>6401-6500</b>  <b>6501-6600</b>  <b>6601-6700</b>  <b>6701-6800</b>  <b>6801-6900</b>  <b>6901-7000</b>  <b>7001-7100</b>  <b>7101-7200</b>  <b>7201-7300</b>  <b>7301-7400</b>  <b>7401-7500</b>  <b>7501-7600</b>  <b>7601-7700</b>  <b>7701-7800</b>  <b>7801-7900</b>  <b>7901-8000</b>  <b>8001-8100</b>  <b>8101-8200</b>  <b>8201-8300</b>  <b>8301-8400</b>  <b>8401-8500</b>  <b>8501-8600</b>  <b>8601-8700</b>  <b>8701-8800</b>  <b>8801-8900</b>  <b>8901-9000</b>  <b>9001-9100</b>  <b>9101-9200</b>  <b>9201-9300</b>  <b>9301-9400</b>  <b>9401-9500</b>  <b>9501-9600</b>  <b>9601-9700</b>  <b>9701-9800</b>  <b>9801-9900</b>  <b>9901-10000</b> </p>	<p> <b>Cancer</b>  <b>Drug Literature Index</b> </p>
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TABLE 1. *Continued*

1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463, 2464, 2465, 2466, 2467, 2468, 2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481, 2482, 2483, 2484, 2485, 2486, 2487, 2488, 2489, 2490, 2491, 2492, 2493, 2494, 2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2511, 2512, 2513, 2514, 2515, 2516, 2517, 2518, 2519, 2520, 2521, 2522, 2523, 2524, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2533, 2534, 2535, 2536, 2537, 2538, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2548, 2549, 2550, 2551, 2552, 2553, 2554, 2555, 2556, 2557, 2558, 2559, 2560, 2561, 2562, 2563, 2564, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2572, 2573, 2574, 2575, 2576, 2577, 2578, 2579, 2580, 2581, 2582, 2583, 2584, 2585, 2586, 2587, 2588, 2589, 2590, 2591, 2592, 2593, 2594, 2595, 2596, 2597, 2598, 2599, 2600, 2601, 2602, 2603, 2604, 2605, 2606, 2607, 2608, 2609, 2610, 2611, 2612, 2613, 2614, 2615, 2616, 2617, 2618, 2619, 2620, 2621, 2622, 2623, 2624, 2625, 2626, 2627, 2628, 2629, 2630, 2631, 2632, 2633, 2634, 2635, 2636, 2637, 2638, 2639, 2640, 2641, 2642, 2643, 2644, 2645, 2646, 2647, 2648, 2649, 2650, 2651, 2652, 2653, 2654, 2655, 2656, 2657, 2658, 2659, 2660, 2661, 2662, 2663, 2664, 2665, 2666, 2667, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 2676, 2677, 2678, 26

Apoptosis is a central biological process, and activation of caspase endoproteases is essential for proper regulation and execution of apoptosis. Because caspases also appear to be central players in several pathological states, there is a practical need within the pharmaceutical research community for facile, reproducible cellular assays for the discovery of compounds that modulate caspase activity. Tandem molecules of green fluorescent protein (GFP) stably expressed within cells can serve as a functionally embedded sensor of protease activity. Using this technology, we have developed a stable cellular system for the screening of agents that modulate activation of the caspase cascade. This assay technology allows for the real-time monitoring of apoptosis in situ, using conventional fluorescent plate reader detection. By applying this assay system to an initial compound screen, small-molecule inducers of cell apoptosis were readily identified. Follow-up pharmacology confirmed that the tandem GFP primary hit using the intracellular GFP assay could be improved and using a conventional, cell lysis-based assay system.



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